J. New York Entomol. Soc. 95(1):14–18, 1987

INFECTIVITY OF EIGHT SPECIES OF ENTOMOGENOUS FUNGI TO THE LARVAE OF THE ELM BARK BEETLE, *SCOLYTUS MULTISTRIATUS* (MARSHAM)

CELESTE HOULE, GEORGE C. HARTMANN, AND S. SALMAN WASTI

Department of Biology, Rhode Island College,
Providence, Rhode Island 02908

Abstract.—Eight species of entomogenous fungi were tested for infectivity to the larvae of *Scolytus multistriatus.* Larvae were inoculated with varying dosages by immersing them in spore suspensions and then incubated at 21°C. High levels of mortality were obtained for most of the test species; *Cordyceps militaris* was not infective under these conditions. Increased spore concentrations of the test fungi did not significantly affect percentage mortality with 7 of the 11 strains of the fungal species tested. Varying temperatures produced complex effects on the host-parasite relationship.

The vector of Dutch elm disease (DED), *Scolytus multistriatus* (Coleoptera: Scolytidae), has been the focus of control procedures because of its accessibility. It has been reported to be susceptible to several species of entomogenous fungi, including *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces farinosus,* and *Verticillium lecanii* (Barson, 1976, 1977; Doberski, 1981a, b). Direct control of the causal fungus, *Ceratocystis ulmi,* has been much more difficult because it is found mainly in the host's xylem vessels. *Pseudomonas syringae* has been reported as an effective bacterial antagonist to *C. ulmi* under both *in vitro* and *in vivo* conditions (Holmes, 1954; Myers and Strobel, 1983; Scheffer, 1983). Fungal growth inhibitors of the DED pathogen have been studied by Gibbs and Smith (1978) and Gemma et al. (1984). The objectives of the present study were to demonstrate the infectivity of entomogenous fungi to the DED vector, *Scolytus multistriatus,* under varying experimental parameters. The complementary infectivity of entomogenous fungi to the insect vector and antagonism to the DED pathogen, *C. ulmi,* were also established. Treatment of diseased elms with these entomogenous fungi would initiate simultaneous activity against the vector and the pathogen.

METHODS

Eleven strains of entomogenous fungal species were evaluated for their infectivity to *Scolytus multistriatus*: *Beauveria bassiana* (Balsamo) Vuill: RS 252, ATCC 26156 (*B. tenella*), and a Russian strain; *Cordyceps militaris* Link: ATCC 26848; *Metarhizium anisopliae* (Metsch.) Sorokin: ATCC 22099; *Hirsutella thompsonii* (Fisher): ATCC 24874; *Nomuraea rileyi* (Farlow) Samson: Missouri strain; *Paecilomyces farinosus* (Dicks. ex Fr.) Brown and Smith: ATCC 24319; *P. fumoso-roseus* (Wise) Brown and Smith: ATCC 16312; *Verticillium lecanii* (Zimm.) Viegas: RH 15-74, ATCC 26854. Stock cultures of each organism were grown on potato-dextrose agar (PDA) with the exception of *N. rileyi* which was grown on Sabouraud maltose agar supplemented with 1% yeast extract (SMYE) and incubated at 24°C for 7 days.

Inoculum for the infectivity studies was prepared by growing each fungal strain on appropriate agar in 10 Petri plates. After 14 days, spores were collected by flooding each of the ten plates with sterile Triton X-100 (0.01%) solution and rubbing off the spores with a bent glass rod. Spore suspensions of each strain were diluted with sterile Triton X-100 (0.01%) solutions to yield the following final concentrations: 10^3 spores/ml, 10^4 spores/ml, and 10^5 spores/ml and used immediately after preparation. Viability of each suspension was confirmed by plating it out on appropriate medium.

Larvae of *Scolytus multistriatus* (Marsham) were obtained from infected elm logs supplied by Dr. John Peacock of the Northeastern Forest Station, Delaware, Ohio. Groups of 30 larvae were washed with 10 ml of sterile Zephiran chloride (1:750) solution for one minute, rinsed with sterile Triton X-100 (0.01%) solution and inoculated with a spore suspension using techniques developed by Doberski (1981a) modified by the insertion of a circular disc, made of fiberglass screening, at the bottom of a syringe barrel to prevent loss of larvae. After inoculation, larvae were transferred to sterile moist chambers; each larva was covered with a sterile vented plastic test tube cap (1.5 cm i.d.). Thirty larvae (10 per moist chamber) were used to replicate each experimental variable tested. Ten larvae inoculated with sterile distilled water served as controls. For each fungal strain, the infectivity of 3 spore concentrations was determined at each of 3 temperatures, 21°C, 25°C, and 30°C.

Inoculated larvae and controls were examined with a binocular dissection microscope at 24 hour intervals. Larvae that failed to respond to prodding with a blunt needle were scored as dead and placed on agar medium containing antibiotics to determine the cause of death. Mortality rates were statistically analyzed using an Analysis of Variance (ANOVA) program. Significant differences were further analyzed using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The percentage mortality of *Scolytus multistriatus* larvae produced by each of the 11 strains of entomogenous fungi at spore concentration of 10^3, 10^4, and 10^5 spores/ml are presented in Table 1. Varying levels of mortality were recorded and *B. bassiana* RS 252, *M. anisopliae* and *P. fumoso-roseus* produced 100% mortality at the highest dosage. Analysis of variance indicated a significant statistical difference between the control and treated larvae. Duncan's (1955) Multiple Range Test showed a significant difference between the controls and the highest dosage. Lowest levels of mortality were produced by *Cordyceps militaris, Hirsutella thompsonii* and *Verticillium lecanii* and while higher dosages demonstrated mortality, the differences among the 3 dosages were not significant.

The mortality response based on treatment with 10^5 spores/ml at varying temperatures is included in Table 2. Analysis of variance showed significant differences between the controls and the larvae exposed to 3 different temperatures. Duncan's (1955) Multiple Range Test demonstrated significant differences between and within the 3 temperature regimes. *B. bassiana* RS 252, Russian strain, and ATCC 26156; *M. anisopliae*; and *P. farinosus* produced a decrease in percentage mortality at 25°C relative to 21°C and 30°C. Mortalities of those larvae inoculated with *C. militaris* and *P. fumoso-roseus* decreased with decreasing temperature. Inoculation with *N. rileyi* and *V. lecanii* ATCC 26854 resulted in a very slight increase in larval mortality

Table 1. Percentage mortality of *S. multistriatus* larvae 10 days after treatment with surface applied suspensions of fungal spores at 21°C.

Test species	Isolate number	Dosage (spores/ml)			
		Control (0)	10^3	10^4	10^5
B. bassiana	RS 252	10	83	86	100
B. bassiana	Russian strain	23	53	80	90
B. bassiana	ATCC 26156	6	23	23	73
C. militaris	ATCC 26848	30	23	40	36
H. thompsonii	ATCC 24874	10	10	16	46
M. anisopliae	ATCC 22099	6	56	66	100
N. rileyi	Ig-Mo	23	66	73	83
P. farinosus	ATCC 24319	6	10	20	76
P. fumoso-roseus	ATCC 16312	0	33	56	100
V. lecanii	ATCC 26854	13	46	50	56
V. lecanii	RH 15-74	10	10	16	46

$F = 14.103$.
$P < 0.05$.

compared to mortality at 21°C and 30°C. *V. lecanii* RH 15-74 caused an increase in percent mortality at both 25°C and 30°C compared to mortality at 21°C.

DISCUSSION

Four species of fungi, *B. bassiana* RS 252, *H. thompsonii, M. anisopliae,* and *P. fumoso-roseus* were significantly more infective when applied at a concentration of 10^5 spores/ml and a temperature of 21°C. Percentage mortalities resulting from the treatment of larvae with the remaining fungal strains did vary significantly with spore concentration. For all strains, percentage mortalities were considerably higher than those of controls at spore concentrations of 10^5/ml and 10^4/ml. Only *C. militaris* was non-infective under these conditions.

Temperature seems to have a variable effect on the host-parasite relationship and results are different from those reported for *S. scolytus.* Barson (1977) noted maximum mortality of *S. scolytus* larvae infected with *B. bassiana* at 25°C and a decline in mortality at 30°C. Doberski (1981b) reported higher levels of mortality when experimental temperatures were increased from 2°C to 20°C. Barson (1977) correlated the decrease in infectivity at 30°C with temperature effects on larval growth, the fungus, and growth enhancement of secondary invaders. Similar factors may account for some of the results reported in this study. *S. multistriatus* may have different optimal temperature requirements from *S. scolytus.* Barson (1977) and Doberski (1981b) reported the long term survival of *S. scolytus* larvae at 5°C and 15°C. In this study, a series of tests at 16°C were discontinued because of high control mortality.

Gemma et al. (1984) have reported on inhibitory interactions between *C. ulmi* and 11 strains of entomogenous fungi. *B. bassiana, M. anisopliae,* and *N. rileyi* were antagonistic to both aggressive and non-aggressive strains of *C. ulmi* under 4 combinations of light and temperature. One of these fungal species, *M. anisopliae,* showed high infectivity to the vector larvae. Entomogenous fungi that are inhibitory to both the causal agent, *C. ulmi,* and infective to the vector provide a multidimensional

Table 2. Percentage mortality of *S. multistriatus* 10 days after treatment with surface applied suspensions of fungal spores (conc. 10^5 spores/ml) at three incubation temperatures.

		Incubation temperature					
		21°C		25°C		30°C	
Test species	Isolate number	Control	Treated	Control	Treated	Control	Treated
B. bassiana	RS 252	10	100	13	53	20	100
B. bassiana	Russian strain	23	90	36	36	33	93
B. bassiana	ATCC 26156	6	73	6	30	30	83
C. militaris	ATCC 26848	30	36	13	80	16	93
H. thompsonii	ATCC 24874	10	46	23	23	—	—
M. anisopliae	ATCC 22099	6	100	30	46	16	100
N. rileyi	Ig-Mo	23	83	23	86	26	83
P. farinosus	ATCC 24319	6	76	6	50	40	80
P. fumoso-roseus	ATCC 16312	0	100	33	73	40	60
V. lecanii	ATCC 26854	13	56	20	76	20	73
V. lecanii	RH 15-74	10	46	80	83	10	100

$F = 29.657$.
$P < 0.05$.

control program against DED, and this possibility is enhanced by the safety of these fungi to nontarget hosts (Donovan-Peluso et al., 1980; Hartmann et al., 1979; Wasti et al., 1980). Determination of the infectivity of the antagonist—entomopathogen under experimental field conditions would confirm the effectiveness of this multidimensional biocontrol program over other unilinear control procedures.

ACKNOWLEDGMENTS

This research was supported by the funds provided by the USDA Forest Service, North Eastern Forest Experiment Station, Broomall, Pennsylvania and a grant from the Faculty Research Fund of Rhode Island College.

LITERATURE CITED

Barson, G. 1976. Laboratory studies on the fungus, *Verticillium lecanii,* a larval pathogen of the large elm beetle (*Scolytus scolytus*). Ann. Appl. Biol. 83:207–214.

Barson, G. 1977. Laboratory evaluation of *Beauveria bassiana* as a pathogen of the elm bark beetle (*Scolytus scolytus*). J. Invertebr. Pathol. 29:361–366.

Doane, C. C. 1959. *Beauveria bassiana* as a pathogen of *Scolytus multistriatus.* Ann. Entomol. Soc. Amer. 52:109–111.

Doberski, J. W. 1981a. Comparative laboratory studies on three fungal pathogens of the elm bark beetle, *Scolytus scolytus*: pathogenicity of *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces farinosus* to larvae and adults of *S. scolytus.* J. Invertebr. Pathol. 37:188–194.

Doberski, J. W. 1981b. Comparative laboratory studies on three fungal pathogens of the elm bark beetle *Scolytus scolytus*: effect of temperature and humidity on infection by *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces farinosus.* J. Invertebr. Pathol. 37:195–200.

Donovan-Peluso, M., S. S. Wasti and G. C. Hartmann. 1980. Safety of entomogenous fungi to vertebrate hosts. Appl. Entomol. Zool. 15:498–499.

Duncan, D. B. 1955. Multiple range and multiple F-tests. Biometrics 11:1–42.

Gemma, J. N., G. C. Hartmann and S. S. Wasti. 1984. Inhibitory interactions between *Ceratocystis ulmi* and several species of entomogenous fungi. Mycologia 76:256–260.

Gibbs, J. N. and M. E. Smith. 1978. Antagonism during the saprophytic phase of the life cycle of two pathogens of woody hosts *Heterobasidion annosum* and *Ceratocystis ulmi.* Ann. Appl. Biol. 89:125–128.

Hartmann, G. C., S. S. Wasti and D. L. Hendrickson. 1979. Murine safety of two species of entomogenous fungi, *Cordyceps militaris* and *Paecilomyces fumoso-roseus.* Appl. Entomol. 14:217–220.

Holmes, F. C. 1954. The Dutch elm disease as investigated by the use of tissue culture, antibiotics, and pectic enzymes. Ph.D. thesis, Cornell University, Ithaca, New York, 150 pp.

Myers, D. F. and G. A. Strobel. 1983. *Pseudomonas syringae* as a microbial antagonist against *Ceratocystis ulmi* in the apoplast of the American elm. Trans. Brit. Mycol. Soc. 73:389–398.

Scheffer, R. J. 1983. Biological control of the Dutch elm disease by *Pseudomonas* species. Ann. Appl. Biol. 103:21–30.

Wasti, S. S., G. C. Hartmann and A. R. Rousseau. 1980. Gypsy moth mycoses by two species of entomogenous fungi and an assessment of their avian toxicity. Parasitology 80:419–424.

Received February 13, 1986; accepted June 17, 1986.